

FBS29 – Capillary Electrophoresis Using the AB 3500/3500xl Genetic Analyzer

Table of Contents

1. Scope
2. Background
3. Safety
4. Materials Required
5. Standards and Controls
6. Procedures
7. Sampling
8. Calculations
9. Uncertainty of Measurement
10. Limitations
11. Documentation
12. References

1. Scope

- 1.1. This procedure is employed to detect amplified product by means of capillary electrophoresis (CE) using the Applied Biosystems® 3500/3500xL Genetic Analyzer.

2. Background

- 2.1. The AB 3500/3500xL Genetic Analyzer is a multi-capillary electrophoresis instrument designed to separate amplified DNA product based on size and record the resulting data in a computerized data file capable of being analyzed using specialized software.
- 2.2. The amplified DNA product is composed of a mixture of differently sized DNA fragments, each containing a fluorescent dye-labeled primer. These primers are specifically designed to differentiate the assortment of amplified loci. As the DNA fragments migrate through the capillary via electrophoresis, a laser light excites the attached fluorescent dye generating an emission of light that is detected and converted to an electrical signal by a charge-coupled device (CCD) camera. The intensity of the resulting signals are converted to relative fluorescence units (rfu) and plotted against the measured time span from a sample's injection to its detection. The data collected corresponding to the amplified DNA fragments is ultimately represented by peaks on an electropherogram.

3. Safety

- 3.1. Wear personal protective equipment (e.g., lab coat, gloves, eye protection), when carrying out standard operating procedures.
- 3.2. Read Safety Data Sheets to determine the safety hazards for chemicals and reagents used in the standard operating procedures.

4. Materials Required

- 4.1. POP 4 Polymer
- 4.2. Hi-Di Formamide (FBR40)

NOTE: Formamide aliquots are kept in the freezer at -20°C and are good for one year from the received date of the stock bottle of Formamide. Once thawed, the aliquots are stored at 4°C and are good for 5 days. Use only deionized formamide. Over time, formamide decomposes into formate. Formate ions are injected preferentially into the capillary, causing a loss in signal intensity.

- 4.3. GlobalFiler™ Allelic Ladder
- 4.4. GeneScan™ 600 LIZ Size Standard

NOTE: Keep the amplified product(s), ladder and size standard protected from direct exposure to light. Excessive exposure can affect fluorescent probes.

- 4.5. Amplified DNA product

5. Standards and Controls

- 5.1. DNA Control 007 is a positive amplification control that is used to evaluate the performance of the amplification and subsequent typing procedures. This control must be included within each CE plate. A positive control does not need to be re-run once it has been properly typed. See FBS30 GlobalFiler™ Data Analysis Using GeneMapper® ID-X, Section 5.1, for expected DNA positive control 007 profile.

- 5.2. The negative amplification control is prepared and processed in parallel with each amplification sample set. A negative control does not need to be re-run once it has been confirmed negative.
- 5.3. The ladder included in each run can also serve as a positive run control. It is used in the analysis of the generated profiles to assign allele calls to the sized peaks of each sample. Additionally, the ladders can be used to demonstrate that the detection equipment and computer software is operating properly by displaying the correct allele sets for each locus and exhibiting consistent peak heights throughout the call range. It is recommended to include one ladder per 1 injection for the 24-capillary instrument and one ladder per 3 injections for the 8-capillary instrument. See FBS30 GlobalFiler™ Data Analysis Using GeneMapper® ID-X, Section 5.1, for a list of the designated alleles that should be detected in the ladder.
- 5.4. If questioned and known samples will be processed on the same 96-well plate, they must be separated by an empty column and must also be injected in separate injections (e.g., for the 24-capillary 3500xL, questioned samples may not be in column “1” and known samples in column “3”.) In addition, questioned samples must be processed on the plate before known samples.

6. Procedures

6.1. Starting the Computer and Instrument

- 6.1.1. Power on the computer and monitor, but do not log in to the Windows® operating system.
- 6.1.2. Once the computer and monitor are powered on, ensure that the instrument door is closed and power on the instrument by pressing the power on/off button on the front. Wait for the green status light.
- 6.1.3. Once status light is green, log on to the Windows® operating system.

6.2. Launching the Applications

- 6.2.1. The Server Monitor should launch automatically. If not, then go to Start → Programs → Applied Biosystems → 3500 → Server Monitor.
- 6.2.2. Once all green checkmarks are displayed on the Server Monitor, click on the 3500 icon on the desktop. The 3500 Series Data Collection Software splash screen appears and will require a log in. Once the User Name and password are entered click **OK**.

6.3. Check System Status in the Dashboard

6.3.1. Perform appropriate maintenance tasks. Refer to FBQ42 and the Sample Tracking and Control Software (STaCS) Instrument/Storage Maintenance log for additional details on maintenance tasks and to determine which, if any, tasks need to be performed.

6.3.2. If applicable, click on the checkmark to mark a task complete.

6.3.3. Check Consumables Status

6.3.3.1. Click **Refresh** to update consumable status.

6.3.3.2. Check the consumables gauges for the number of injections, samples, or days remaining for each. If consumables have expired or if buffer fill level is too low, replenish as directed below. (See also 3500/3500xL Genetic Analyzer User Guide).

6.3.4. Changing the Polymer

6.3.4.1. Remove the polymer pouch from the refrigerator and allow to come to room temperature.

6.3.4.2. Enter the appropriate information into STaCS.

6.3.4.3. In the Dashboard, click **Wizards**, then click **Replenish Polymer** and follow the prompts.

NOTE: A polymer pouch installed on an 8-capillary (3500) instrument cannot be used on a 24-capillary (3500xL) instrument or vice versa. Doing so may result in a lower number of samples/injections than specified.

6.3.5. Changing the Anode Buffer Container (ABC)

6.3.5.1. Remove the ABC from the refrigerator and allow to come to room temperature.

6.3.5.2. Enter the appropriate information into STaCS.

6.3.5.3. Verify that the seal is intact. Do not use if the buffer level is too low or the seal has been compromised.

6.3.5.4. Invert the ABC, and then tilt it slightly to move most of the buffer to the larger side of the container. The smaller side of the container should contain <1 mL of the buffer.

6.3.5.5. Verify that the buffer is at the fill line.

6.3.5.6. Peel off the seal at the top of the ABC and with the radio frequency identification (RFID) label pointed toward the instrument, place the ABC into the anode-end of the instrument, below the pump. Position the anode in the

large chamber of the ABC, then push the ABC up and back to install.

6.3.5.7. Close the instrument door to re-initialize.

6.3.5.8. In the Dashboard, click **Refresh**, then check the Quick View section for updated status.

6.3.6. Changing the Cathode Buffer Container (CBC)

6.3.6.1. Remove the CBC from the refrigerator and allow to come to room temperature.

6.3.6.2. Enter the appropriate information into STaCS.

6.3.6.3. Wipe away condensation on the CBC exterior with a Kimwipe.

6.3.6.4. Check that seal is intact. Do not use if buffer level is too low or seal has been compromised.

6.3.6.5. Tilt the CBC back and forth gently and carefully to ensure the buffer is evenly distributed across the top of the baffles.

6.3.6.6. When ready to install CBC, place the container on a flat surface and peel off the seal.

6.3.6.7. Wipe off any buffer on top of the CBC with a Kimwipe.

6.3.6.8. Place the appropriate septum on each side of the CBC.

6.3.6.9. Ensure the instrument door is closed, then click the **Tray** button to move the autosampler to the front position.

6.3.6.10. With the tab facing you and the RFID tag to the right, install the CBC on the autosampler. When properly installed, the CBC tabs will click as you snap them into place on the autosampler.

6.3.6.11. Close the instrument door to retract the autosampler.

6.3.6.12. In the Dashboard, click **Refresh**, then check the Quick View section for updated status.

6.4. Preparing a Run:

6.4.1. Creating a Setup Sheet

6.4.1.1. Create a set-up sheet within STaCS. It is recommended to include one ladder per 1 injection for the 24-capillary instrument and one ladder per 3 injections for the 8-capillary instrument.

- 6.4.1.2. The amount of each component needed in the Formamide/LIZ mixture will be calculated upon entry into the STaCS documentation. The worksheet will calculate the appropriate volume of each component.

of Samples x 9.6 µl Hi-Di Formamide

of Samples x 0.4 µl GS600 LIZ Standard

NOTE: The number of reactions will include enough of the Formamide/LIZ mixture to complete an injection/run (8 or 24 wells). Alternatively, Formamide may be used to fill the remaining wells in an injection. An empty well may cause damage to the capillary.

NOTE: Extra reactions or an additional percentage of the number of reactions can be added to the calculations in order to account for any volume lost during pipetting.

6.4.2. Pre-heating the Oven

OPTIONAL: This is an optional but recommended step. If this step is not done, the instrument run will not begin until the oven is 60°C.

- 6.4.2.1. In the Dashboard, under Instrument Information, set the oven temperature and then click **Start Pre-heat**.

6.4.3. Sample Preparation

- 6.4.3.1. Retrieve Hi-Di Formamide, Size Standard and Allelic Ladder. Enter the appropriate information into STaCS.
- 6.4.3.2. Vortex and pulse spin all of the reagents.
- 6.4.3.3. Retrieve a 1.5ml or 2.0ml tube and label. (If running a large quantity of samples, the Formamide/Size Standard mixture can be prepared in a V-bottom basin.)
- 6.4.3.4. Add the required amount of each component to the tube (or V-bottom basin).
- 6.4.3.5. Vortex and pulse spin the Formamide/Size Standard mixture if prepared in a tube. If prepared in a V-bottom basin, be sure to thoroughly mix.
- 6.4.3.6. Allow the amplified product to equilibrate to room temperature. Spin all of the tubes/plates to ensure amplified product is concentrated in the bottom of each tube/well.
- 6.4.3.7. Obtain a 96-well plate and properly label it with the plate name, date, and initials. Additional markings can be

made on the plate to indicate rows and columns at the discretion of the analyst.

- 6.4.3.8. Aliquot 10 µl of the Formamide/Size Standard mixture into each sample well. Be sure to fill ALL of the wells associated with the injection/run (8 or 24 wells) with Formamide or Formamide/Size Standard mixture. Capillaries should not attempt to inject empty wells.
- 6.4.3.9. Following the STaCS 3500 Setup Sheet, aliquot 1µl of Allelic Ladder or 1µl amplified product to the appropriate wells.
- 6.4.3.10. Obtain a 96-well septum and check to be sure all septum holes are open. Seal the plate by laying the septum flat on the plate, aligning the wells, and pressing down. Be certain that the septum fits securely and completely on the plate.
- 6.4.3.11. Centrifuge the plate at 3000 rpm (1811 rcf) for approximately 30 seconds to 1 minute to ensure all liquid is concentrated in the bottom of each well.
- 6.4.3.12. Turn on the thermal cycler (thermal cycler may be pre-heated). Place the plate in the thermal cycler.

NOTE: Do not close the lid because the septa may melt to the plate.

Select and start the appropriate thermal cycling program ("denature") in order to denature the plate. The method on the screen should correspond to the following:

HOLD	95°C	3:00 MIN
HOLD	4°C	3:00 MIN
HOLD	4°C	FOREVER

- 6.4.3.13. When denaturation is complete, the plate will be left at 4°C in the thermal cycler (or placed in the fridge) until it is ready for assembly onto the autosampler.

NOTE: The fluorescent dyes attached to the primers are light-sensitive. Be sure to minimize their exposure time by storing all samples, allelic ladder and LIZ size standard away from light.

6.5. Performing the Run

6.5.1. Use the following steps as a guide.

- 6.5.1.1. Construct the plate assembly by placing the sample plate in the plate base and snapping on the retainer. Ensure that the notches line up and that the retainer holes are properly aligned with the septum holes.
- 6.5.1.2. After instrument doors are closed, press the **Tray** button on the front of the 3500/3500xL. Wait for the autosampler to finish moving to the front and then open the instrument doors.
- 6.5.1.3. Place the plate assembly on the autosampler. The notched area on the base will be toward the back of the instrument. Press gently but firmly to be certain the tray is flat and properly placed on the autosampler.
- 6.5.1.4. Check the septa on the CBC reservoir to be certain that they are flush.
- 6.5.1.5. Close the doors and allow the autosampler to completely move into the home position before continuing to next step.
- 6.5.1.6. Select **Create New Plate** icon from 3500 Series Home screen.
NOTE: This will assign the Assay type “Casework” to the samples:

3500xL injection: 1.2kV for 24 seconds

3500 injection: 1.2kV for 15 seconds
- 6.5.1.7. On the “Define Plate Properties” screen, correctly fill in the Plate name and ensure other Plate details are correct. When Complete click the **Assign Plate Contents** icon.
- 6.5.1.8. Once on the “Assign Plate Contents” screen use the import icon to select the previously generated STaCS text file for the associated run.
- 6.5.1.9. **NOTE:** By clicking the “Table View” tab, sample types can be designated for all wells in run.
- 6.5.1.10. In the “Assign Plates for Run” screen, click **Link Plate for Run**.

NOTE: By default, plate A position is selected.

- 6.5.1.11. Access the “Load Plates for Run” screen.
- 6.5.1.12. Review the consumables information and the calibration information and ensure the status is acceptable for a run.
- 6.5.1.13. Enter a Run Name.
- 6.5.1.14. As needed, click **Switch Plates** to assign the plate to the other position in the autosampler.
- 6.5.1.15. Click **Create Injection List** to view the Run schedule for the plate.
- 6.5.1.16. Click **Start Run**.

6.6. Exporting Data:

- 6.6.1. In the Dashboard, navigate to the “Workflow” and “Monitor Run” tabs. If there are no reinjections to perform, click **Resume Run**. If there are reinjections, proceed to Section 6.7.
- 6.6.2. Once the run has been completed select “My Computer” on the desktop > “AB SW & DATA (D:)” > “Applied Biosystems” > “3500” > “DATA”. Alternatively, click on the data collection shortcut.

NOTE: The **Resume Run** button must be clicked at the end of each run to ensure proper completion of the run files. Failure to do so may result in the replacement of run folders and/or run files from the previous plate.
- 6.6.3. Locate the project folder, copy and paste the project to the designated storage location (i.e., share drive or thumb drive).

6.7. Reinjects:

- 6.7.1. Reinjects can only be performed when the run has not yet been completed. When viewing the Dashboard on the “Workflow” and “Monitor Run” tabs, if the **Resume Run** button is grayed out, reinjects cannot be performed.
- 6.7.2. On the Dashboard, navigate to the “Workflow” and “Monitor Run” tabs (top and side bars, respectively).
- 6.7.3. At the bottom of the screen, click **Review Results**.
- 6.7.4. From the “Samples View” table, select the samples identified for reinjection, as well as the associated ladders.
 - 6.7.4.1. Multiple samples can be selected by holding down the “control” key.
 - 6.7.4.2. If multiple plates were included in the initial run, ensure you are selecting the samples from the correct plate. The

plate name is listed in the “plate name column” on the far right of the screen.

6.7.5 Click **Reinject** at the top of the screen.

6.7.5.1 A screen will pop up. Verify that the “reuse the existing protocol” and “following all injections” are selected and click **OK**.

6.7.6 From the “Monitor Run” tab, verify that the reinjects have been added after the previously scheduled injections.

6.7.7 Click **Resume Run**.

6.7.8 Once the run has completed follow the steps as outlined in Section 6.6.

7. Sampling

7.1. Not applicable

8. Calculations

8.1. Not applicable

9. Uncertainty of Measurement

9.1. Not applicable

10. Limitations

10.1. Not applicable

11. Documentation

11.1. Applicable STaCS documentation

12. References

12.1. Applied Biosystems 3500/3500xL Genetic Analyzer User Guide, Applied Biosystems, Foster City, CA.

12.2. FBR40 - Hi-Di Formamide (Current Version)

- 12.3. FBS28 - PCR Amplification Using the GlobalFiler™ Kit (Current Version)
- 12.4. FBS30 - GlobalFiler™ Data Analysis Using GeneMapper® ID-X (Current Version)
- 12.5. FBQ41 - Quality Control of GlobalFiler™ PCR Amplification Kits (Current Version)
- 12.6. FBQ42 - Maintenance of the AB 3500/3500xL Genetic Analyzer (Current Version)
- 12.7. Forensic Science Laboratory Quality Assurance Manual (Current Version)
- 12.8. Forensic Biology Unit Quality Assurance Manual (Current Version)
- 12.9. FSL Departmental Operations Manuals (Current Versions)
- 12.10. FSL Laboratory Operations Manuals (Current Versions)